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Metabolites of $PGF_{2\alpha}$ in Blood Plasma and Urine as Parameters of $PGF_{2\alpha}$ Release in Cattle

By *Samar Basu, Hans Kindahl, Denis Harvey* and *Keith J. Betteridge*

Basu S., H. Kindahl, D. Harvey and K. J. Betteridge: Metabolites of $PGF_{2\alpha}$ in blood plasma and urine as parameters of $PGF_{2\alpha}$ release in cattle. Acta vet. scand. 1987, 28, 409–420. – The metabolism of $PGF_{2\alpha}$ in cattle results initially in the formation of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (15-ketodihydro-$PGF_{2\alpha}$) and later the 11-ketotetranor PGF metabolites. Both types of metabolites appear in the peripheral circulation and finally the 11-ketotetranor PGF metabolites are found in large quantities in the urine in a species-related pattern. Several approaches can be made to the quantitative analysis of $PGF_{2\alpha}$ release during reproductive studies. First, assay of the 15-ketodihydro-$PGF_{2\alpha}$ metabolite in the peripheral circulation; second, analysis of the longer-lived 11-ketotetranor PGF metabolites in the peripheral circulation; and finally analysis of the latter metabolites in the urine. The antibodies used in radioimmunoassays of both types of metabolites of $PGF_{2\alpha}$ were found to be specific and the results agree well with those obtained earlier by mass spectrometric analysis. The assay of 11-ketotetranor PGF metabolites was used to study the excretion of urinary metabolites in the cow after i.v. infusion of $PGF_{2\alpha}$ and also during the normal estrous cycle and early pregnancy. These studies suggest that 11-ketotetranor PGF metabolites in cow urine serve as a good parameter of $PGF_{2\alpha}$ release, especially for long-term studies, but when a precise pattern of $PGF_{2\alpha}$ release is required, measurement of 15-ketodihydro-$PGF_{2\alpha}$ levels in frequently collected plasma samples is preferable.

radioimmunoassay; prostaglandin; metabolism; estrous cycle; early pregnancy; cow.

Introduction

During the past two decades the biological importance of prostaglandins has been studied intensively and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a product of arachidonic acid metabolism, has proved to be of particular interest in the field of reproduction (*Horton & Poyser* 1976). The primary $PGF_{2\alpha}$ has an extremely short half-life and is metabolised to biologically inactive 15-ketodihydro-$PGF_{2\alpha}$ which is found in the peripheral circulation (for reviews, see *Samuelsson et al.* 1975, 1978). This involves dehydrogenation at the C_{15} position by 15-hydroxyprostaonate dehydrogenase and reduction of Δ^{13}-double bond by Δ^{13}-reductase. Subsequently this 15-ketodihydro-$PGF_{2\alpha}$ is degraded by two steps of β-oxidation and also by ω-oxidation to a number of other metabolites. Many of these are 11-ketotetranor PGF metabolites which appear in plasma and are later excre-

ted in urine in a species-related pattern, often in large quantities (*Granström & Kindahl* 1982a, *Granström et al.* 1982, *Goff et al.* 1984). Due to this rapid metabolism (*Hamberg & Samuelsson* 1971, *Granström* 1972) and also the artefactual formation of $PGF_{2\alpha}$ during collection of blood samples (*Samuelsson* 1974), measurement of $PGF_{2\alpha}$ itself in the peripheral circulation is not a suitable parameter of its physiological activity. Having a longer half-life than the parent compound and not being formed artefactually during collection and handling of samples, the initial metabolite 15-keto-dihydro-$PGF_{2\alpha}$ in plasma has been widely used as a good parameter of $PGF_{2\alpha}$ release. However, its usefulness is limited to studies which involve frequent sampling, otherwise pulsative peaks of short duration might easily be missed. In this study, parts of which were previously presented in brief (*Basu et al.* 1984), a radioimmunoassay has been established to measure 11-ketotetranor PGF metabolites in plasma and urine from cattle and used in an investigation of the best parameters for the measurement of $PGF_{2\alpha}$ release in this species.

Materials and methods

Chemicals

Tris-HCl buffer 0.05M, pH 7.8 containing lmM EDTA was used as the radioimmunoassay (RIA) buffer. Bovine γ-globulin 0.25 % was prepared in RIA buffer. The labelled ligand [5,6,8,9,11,12,14,(n)-$^3H_7$]-15-keto-13-14-dihydro-$PGF_{2\alpha}$ (specific activity, 160 Ci/mmol and in some experiments 80 Ci/mmol) was obtained from Amersham Laboratories, England. Unlabelled standard of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ was obtained from the Upjohn Company, Kalamazoo, Michigan, USA. Polyethylene glycol (MW 4000) was purchased from Kebo-lab, Stockholm.

Biological samples

Blood and urine samples were collected from cattle during exogenous administration of unlabelled $PGF_{2\alpha}$ and the separated plasma and aliquots of urine were stored frozen until analysis. Urinary samples were also collected to monitor the endogenous $PGF_{2\alpha}$ release during the estrous cycle and early pregnancy (*Harvey et al.* 1984) and, in 1 case, throughout pregnancy and until about 20 days postpartum.

In 1 heifer, blood samples were taken every hour during the luteolytic period. The samples, which had earlier been analysed for their content of 15-ketodihydro-$PGF_{2\alpha}$ (*Kindahl et al.* 1976b), were reanalysed for 11-ketotetranor PGF metabolites (see below).

Radioimmunoassay for 15-ketodihydro-$PGF_{2\alpha}$

The plasma samples from the infusion experiment were analysed according to the method described by *Betteridge et al.* (1984). All other plasma samples from the luteolytic period, urinary samples from the infusion experiment and cross-reactivity tests against 11-ketotetranor PGF metabolites were analysed as described previously with some modifications (*Granström & Kindahl* 1982b). Briefly, by using a series of standards of unlabelled 15-ketodihydro-$PGF_{2\alpha}$ diluted in RIA buffer ranging from 4 to 1024 pg/0.1 ml, a standard curve was obtained. A working titre of the antibody was kept at 1:3200, making the final dilution in the tube 1:22,400. The radioactive tracer was diluted in RIA buffer to 75,000 dpm/ml. The practical procedure for analysing 15-ketodihydro-$PGF_{2\alpha}$ in plasma and urine was analogous to that described below for 11-ketotetranor PGF metabolites.

Radioimmunoassay for 11-ketotetranor PGF metabolites in plasma and urine

Preparation of labelled ligand: 5α,7α-Dihydroxy-11-ketotetranor-[2,4,5,7,8,10(n)-^{3}H]-prostanoic acid was prepared in vitro by β-oxidation of 15-keto-13,14-dihydro [$^3H_7$]-$PGF_{2\alpha}$ (specific activity 80 Ci/mmol), using washed rat liver mitochondria and appropriate cofactors (*Hamberg* 1968, *Granström & Kindahl* 1976). The β-oxidised products were separated by high performance liquid chromatography (HPLC), using the solvent system methanol/water/acetic acid (60:40:0.01, v/v/v). The resulting tetranor compounds were found in two peaks representing their open and δ-lactone forms. The antibody (see below) binds the radioactive material in the two peaks equally well after an equilibration period of at least two days in RIA buffer. The radioactive tracer was diluted in buffer to 50,000 dpm/ml as working solution and stored at + 4°C.

Preparation of standard: Standard was prepared by β-oxidation of 15-keto-13,14-dihydro [17,18-^{3}H]-$PGF_{2\alpha}$ (100 μg, specific activity 0.3 Ci/mol) in vitro to yield 5α,7α-dihydroxy-11-ketotetranorprostanoic acid. The basic procedure was the same as explained for preparation of labelled ligand.

Preparation of the antibody: 5α,7α-Dihydroxy-11-ketotetranorprostane-1,16-dioic acid was kept in glacial acetic acid at room temperature to induce formation of a δ-lactone form (*Granström & Kindahl* 1976). Later the free ω-carboxyl was coupled to bovine serum albumin (BSA) using N,N'-carbonyl-diimidazole. The lyophilized conjugate was dissolved in water and emulsified with Freund's complete adjuvant and later injected into 2 rabbits subcutaneously and intradermally in the flank area 3 times at 1 week intervals. A fourth booster injection was given after a rest period of two months. The antibody titre reached a maximum about 10 days after the booster injection at which time the animals were bled.

Practical procedure for analysing 11-ketotetranor PGF metabolites in the plasma and urine: For peripheral plasma, 50 μl samples were used without extraction; urine was diluted 100- and 500-fold in RIA buffer and 50 and 100 μl aliquots were taken in duplicate for radioimmunoassay. To a series of standard tubes (ranging from 0.8 to 100 pg/0.1 ml 5α,7α-dihydroxy-11-ketotetranorprostanoic acid) and unknown samples, 0.2 ml 0.25 % bovine γ-globulin and Tris buffer were added to a final volume of 0.5 ml. Finally 0.1 ml of antibody dilution (working titre 1:1,600, final dilution in the tube 1:11,200) and 0.1 ml of radioactive tracer were added, the tubes were shaken carefully, and incubated overnight at + 4°C. Incubation for 5–48 h in the cold or at room temperature causes no significant difference in the results. Separation of free and antibody-bound fractions was performed by using 25 % polyethylene glycol dissolved in distilled water to precipitate the antibody-bound fraction. Addition of 0.7 ml polyethylene glycol was done by a Cornwall syringe while the tubes were kept in an ice bath. The tubes were then shaken vigorously for 15–20 s. After centrifugation for 1 h at 1400 × g and + 1°C, 1 ml of the supernatant containing the free fraction was removed and diluted in 1 ml distilled water using an LKB Diluter 2075. Ten ml of scintillation cocktail (Instagel) was added and the vials were well shaken. The initially formed emulsion becomes clear after keeping the vials in the cold for 12 h. Radioactivity was counted in a Packard Tri-Carb β-Scintillation counter furnished with automatic

external channel standardization. All calculations were made off-line with a programmed computer as a function of logit versus log amount of unlabelled prostaglandin metabolite.

Results and discussion

Properties of the assay for 11-ketotetranor PGF metabolites

Evaluation of the assay: Intra-assay and inter-assay coefficients of variation were 6.3 to 10.1 % and < 12 %, respectively. For urinary analysis, linearity was determined from 50 and 100 µl samples of diluted urine which gave mean values of 30.5 and 30.7 pg/ml, respectively (n = 50), for basal levels during the estrous cycle. A similar test using plasma samples of 50, 100 amd 200 µl also showed linearity.

Specificity of the antibodies: The metabolic pathways of primary $PGF_{2\alpha}$ in the cow are shown in Fig. 1 (*Granström & Kindahl* 1982a). The finally degraded metabolites consist of 3 tetranor metabolites which are found both in plasma and urine. The antibody against 5α,7α-dihydroxy-11-keto-tetranorprostane-1, 16-dioic acid was utilized for determination of these finally degraded shorter metabolites (*Granström & Kindahl* 1976, 1982c). The ω-carboxyl group of this compound was selectively coupled to the amino group of bovine serum albumin and so the resulting antibody cannot discriminate between metabolites that differ only at the ω-end. The specificity of anti-5α,7α-dihydroxy-11-ketotetranorprostane-1, 16 dioic acid was determined against various $PGF_{2\alpha}$ metabolites appearing in the urine (*Granström & Kindahl* 1976). When 5α,7α-dihydroxy-11-ketotetranorprostanoic acid was used as standard in the present experiment (set to 100 % cross-reaction) metabolites 1 and 2 in Fig. 1 cross-reacted about 100 %. Metabolite no. 3, the main metabolite in cow urine, cross-reacted about 60 % due to the presence of an hydroxyl group instead of a keto group at the C-11 position in the metabolite. Thus the antibody efficiently recognizes all the

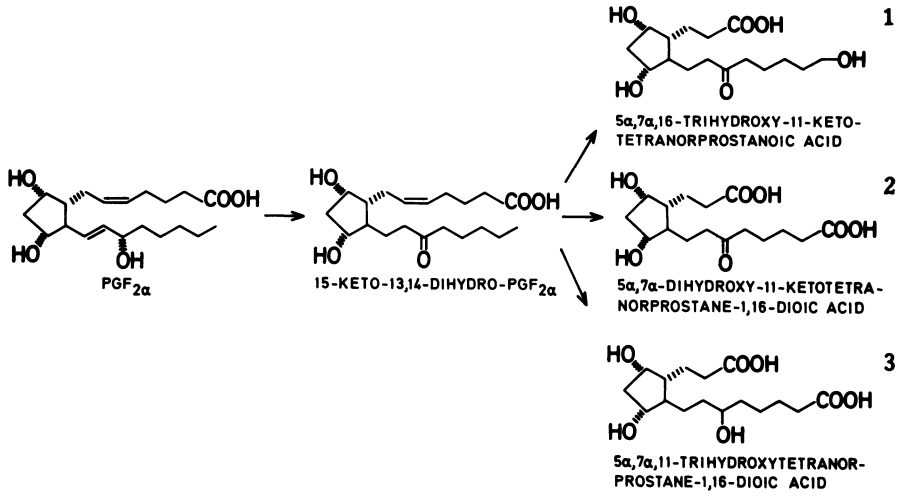

Figure 1. Steps in the metabolism of primary $PGF_{2\alpha}$ in the cow (*Granström & Kindahl* 1982a).

tetranor metabolites of $PGF_{2\alpha}$ that predominate in bovine urine.

Since the 15-ketodihydro-$PGF_{2\alpha}$ metabolite occurs in high concentrations in bovine plasma (*Kindahl et al.* 1976a) and could possibly be excreted into the urine and interfere in the assay of 11-ketotetranor PGF metabolites, it was necessary to check the cross-reactivity of the antibody to the 15-ketodihydro-$PGF_{2\alpha}$ metabolite. The results are shown in Fig. 2. At 50 % displacement a cross-reactivity of 0.0054 % was observed, with a very nominal change seen at lower and higher levels of the standard curve. Thus the influence of 15-ketodihydro-$PGF_{2\alpha}$ on measurement of 11-ketotetranor PGF metabolites is probably negligible.

Properties of the assay for 15-ketodihydro-$PGF_{2\alpha}$

Specificity of the antibody: If, for practical reasons, the 15-ketodihydro-$PGF_{2\alpha}$ assay is to be used for both plasma and urine as a measure of $PGF_{2\alpha}$ secretion, sufficient cross-reaction between the antibody against 15-ketodihydro-$PGF_{2\alpha}$ and the 11-ketotetranor PGF metabolites would be required. Fig. 3 shows the extent of this cross-reactivity. At 50 % displacement cross-reactivity was 0.7 %; at 10 % displacement about 7 %; at 90 % displacement about 0.2 %. These results, indicate the possibility that very high levels of tetranor metabolites could influence the 15-ketodihydro-$PGF_{2\alpha}$ assay. Such a situation does occur after exogenous administration of $PGF_{2\alpha}$ when large amount of β-oxidised products are excreted into the urine. However, the cross-reaction is too low to make this antibody useful for the analysis of 11-ketotetranor PGF metabolites in physiological studies.

Kinetics of prostaglandin $F_{2\alpha}$ metabolites

The results of concurrent assays, applied to

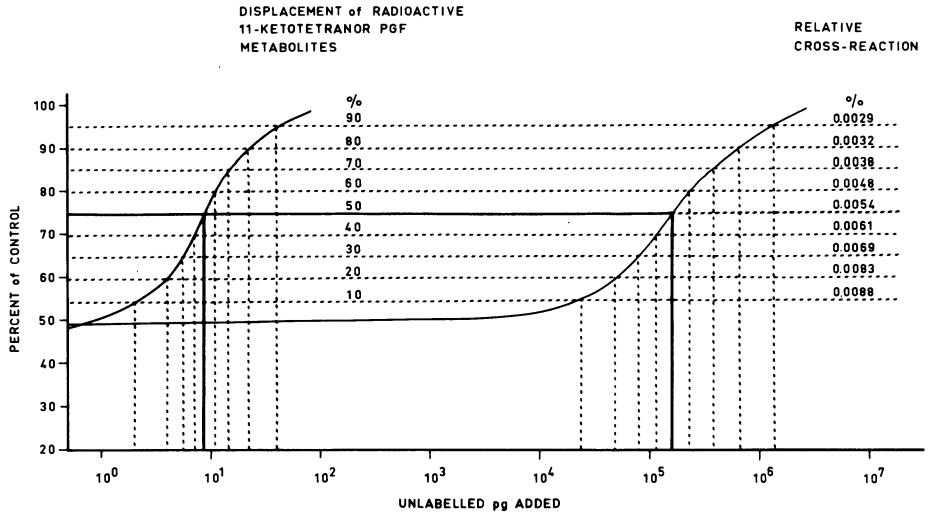

Figure 2. Specificity of the binding by anti-5α,7α-dihydroxy-11-ketotetranorprostane-1,16-dioic acid of unlabelled 5α,7α-dihydroxy-11-ketotetranorprostanoic acid (left curve and 15-ketodihydro-$PGF_{2\alpha}$ (right curve). The curves are compared at various displacement levels. At 50 % displacement 160,000 pg of unlabelled 15-ketodihydro-$PGF_{2\alpha}$ is required to cause the same displacement of the radiolabelled molecule as 8.6 pg of the proper compound (cross-reaction of 0.0054 %).

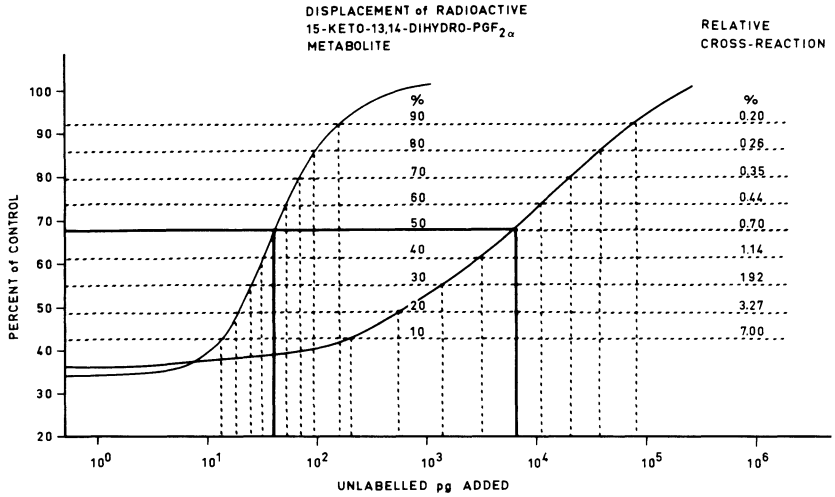

Figure 3. Specificity of the binding by anti-15-ketodihydro-$PGF_{2\alpha}$ of unlabelled 15-ketodihydro-$PGF_{2\alpha}$ (left curve) and 5α,7α-dihydroxy-11-ketotetranorprostanoic acid (right curve). The curves are compared at various displacement levels. At 50 % displacement 5,700 pg of unlabelled 5α,7α-dihydroxy-11-ketotetranorprostanoic acid is required to cause the same displacement of the radiolabelled molecule as 40 pg of the proper compound (cross-reaction of 0.70 %).

urine and plasma before, during and after infusion of unlabelled prostaglandin $F_{2\alpha}$ into a oophorohysterectomized heifer are shown in Fig. 4. The basal level of 15-ketodihydro-$PGF_{2\alpha}$ in urine was 2.5 ng/ml whereas that of the 11-ketotetranor PGF metabolites was approximately 6 times higher (15.0 ng/ml). The basal level of 15-ketodihydro-$PGF_{2\alpha}$ in plasma was about 125 pg/ml. When 125 μg of $PGF_{2\alpha}$ was infused, 15-ketodihydro-$PGF_{2\alpha}$ metabolite increased up to 4.3 ng/ml and 11-ketotetranor PGF metabolites up to 143 ng/ml in urine, which were about 1.7 and 9.5 times increase from their basal levels, respectively. Thus, the increase in 11-keto metabolites was about 5.6 times greater than the increase in the 15-keto metabolite. When 500 μg of $PGF_{2\alpha}$ was infused, the levels of 15-keto metabolite rose to a maximum 21.0 ng/ml and 11-keto metabolites to 740 ng/ml in urine, which were about 8.4 and 49.3 times increase from their basal levels, respectively. Thus the maximal concentrations 11-ketotetranor PGF metabolites attained were about 5.9 times higher than those of the 15-ketodihydro-$PGF_{2\alpha}$ metabolite. Overall, 15-ketodihydro-$PGF_{2\alpha}$ metabolite concentrations during these infusions were about 5.8 times lower than those of the 11-ketotetranor PGF metabolites in the urine. Furthermore, the peaks of 11-ketotetranor PGF metabolites appeared about 30–40 min later than those of 15-ketodihydro-$PGF_{2\alpha}$ metabolite in urine. 15-Ketodihydro-$PGF_{2\alpha}$ level in plasma increased to over 300 and 1200 pg/ml, respectively, after the 2 infusions. The peak levels in 15-keto metabolite in plasma were found about 30–40 min before the peaks of 11-keto metabolites in urine. Although the concentrations of the 15-keto metabolite determined in urine could probably to some extend be due to the cross-reaction of the 11-ketotetranor PGF metabolites in the as-

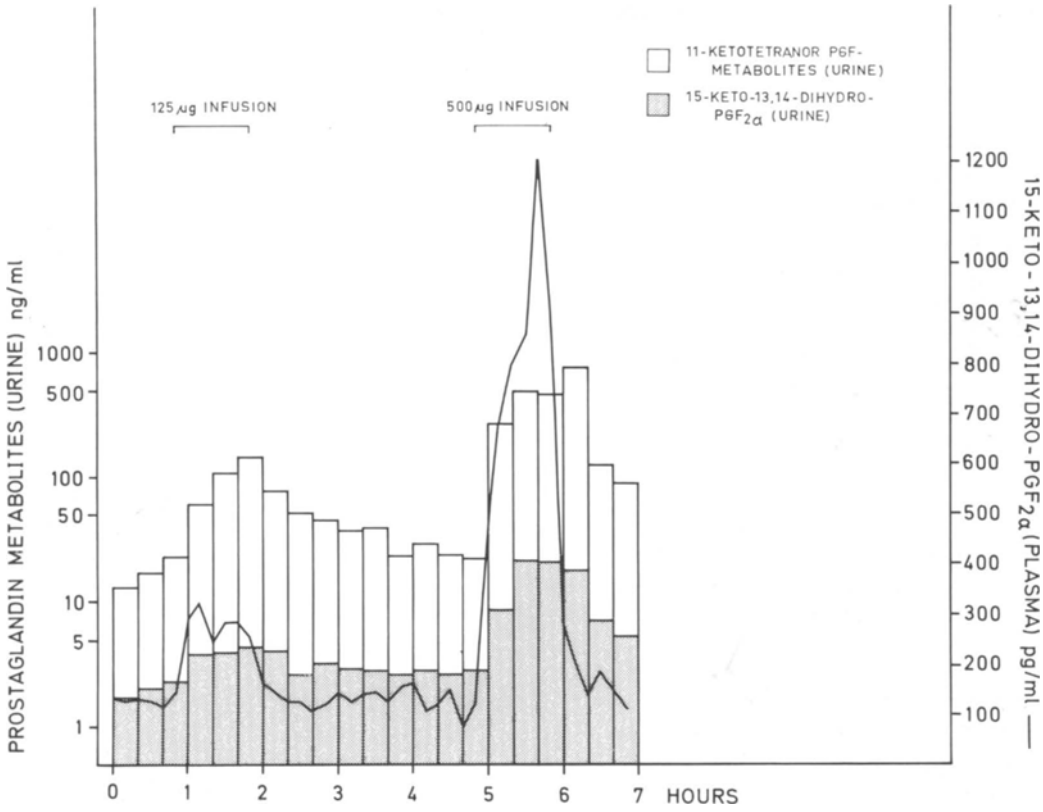

Figure 4. Appearance and disappearance of 15-ketodihydro-$PGF_{2\alpha}$ in plasma and urine and 11-ketotetranor PGF metabolites in urine after i.v. infusion of unlabelled $PGF_{2\alpha}$ in a oophorohysterectomized heifer. The exogenous $PGF_{2\alpha}$ was infused over two different 1-hour periods at the rates of 125 µg/hour and 500 µg/hour, as indicated. Note that a logarithmic scale is used for both $PGF_{2\alpha}$ metabolites in urine; a linear scale for 15-ketodihydro-$PGF_{2\alpha}$ in plasma.

say, it is probable that these levels represent true excretion of 15-ketodihydro-$PGF_{2\alpha}$ metabolite into the urine. This contention is supported by the fact that a small interval exists between the times of appearance of these two metabolites of $PGF_{2\alpha}$ in the urine. In conclusion, a very low level of 15-keto metabolite is monitored in the urine after exogenous administration of $PGF_{2\alpha}$ and assay of the 11-ketotetranor PGF metabolites is the parameter of choice for following $PGF_{2\alpha}$ metabolism and excretion by this route. For example, Fig. 5, an extension of Fig. 7 from *Harvey et al.* (1984), depicts the levels from one cow followed throughout pregnancy and for about 20 days postpartum.

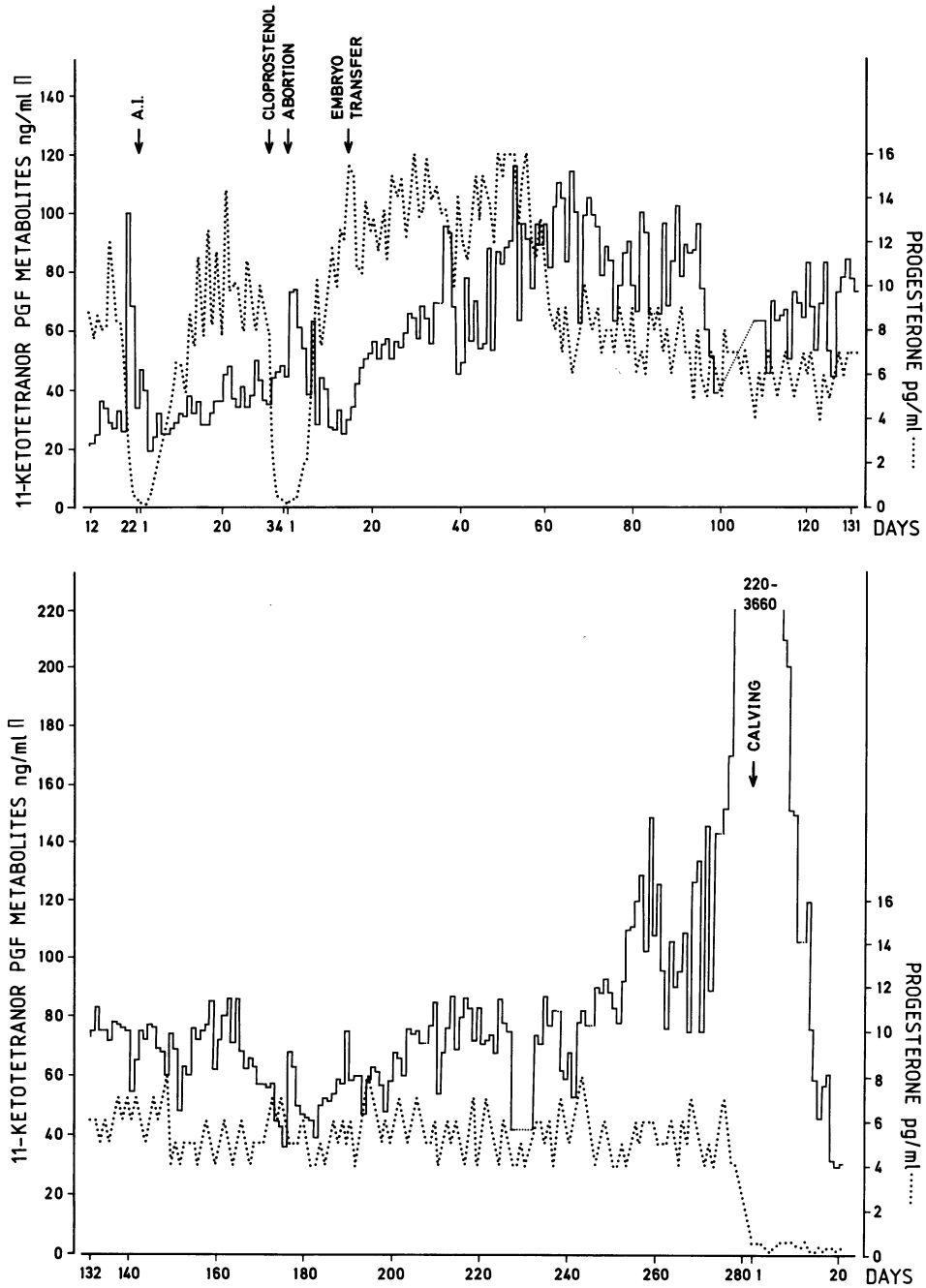

Figure 5. Concentrations of urinary 11-ketotetranor PGF metabolites and peripheral plasma levels of progesterone (measured according to *Betteridge et al.* 1977) in a heifer during estrous cycle, early pregnancy, following induced abortion, after embryo transfer at day 15, throughout pregnancy, parturition and until about 20 days postpartum. Urinary metabolite values were not corrected for variation in glomerular filtration rate.

Levels of prostaglandin metabolites in plasma during luteolysis

The concentrations of both 15-ketodihydro-$PGF_{2\alpha}$ and 11-ketotetranor PGF metabolites found in bovine plasma during the luteolytic period are shown in Fig. 6. As can be seen, it was the 15-keto metabolite that produced the highest peaks; the peak levels of 11-ketotetranor PGF metabolites were lower and slightly more sustained. Generally, when comparing the peak levels, the 11-ketotetranor PGF metabolites appeared one sample (1 h) later than 15-keto metabolite. *Granström & Kindahl* (1982a) studied the appearance and disappearance of 15-ketodihydro-$PGF_{2\alpha}$ and 11-ketotetranor PGF metabolites in peripheral plasma after exogenous administration of $PGF_{2\alpha}$ in the cow and found that levels of 11-keto metabolites never exceeded the levels of 15-keto metabolite in plasma, as was true during luteolysis in this study. With their more frequent sampling, *Granström & Kindahl* (1982a) observed a time difference of about 15 min between peak levels of the two metabolites in plasma. From Fig. 7 the lengths of periods (peaks) during which the basal levels of each metabolite of $PGF_{2\alpha}$ were exceeded by more than +2 SD of their respective mean basal levels were calculated. The mean basal level was calculated from the sample collected the day before the first peak. It was seen that there were peaks of 11-keto metabolites during 62.4 % of the time, and of 15-keto metabolite for about 52.7 % of the time, for the 3 days (approximately) of luteolysis. Peaks of one or both of the metabolites extended over 69.2 % of the luteolytic period, showing that measurement of both metabolites in the same plasma samples increases the chances of detecting short-lived releases of $PGF_{2\alpha}$.

In conclusion, this study corroborates the value of measuring the 15-ketodihydro-$PGF_{2\alpha}$ metabolite in plasma and the 11-ketotetranor PGF metabolites in plasma and urine as parameters of the release of primary $PGF_{2\alpha}$ in cattle. Collection of urine for measurement of 11-ketotetranor PGF metabolites makes it possible to perform long-term studies of $PGF_{2\alpha}$ release in the cow using daily sampling rather than the much more arduous frequent blood sampling which is necessary if release is to be detected by measurement of metabolites in plasma (*Harvey et al.* 1984). Although this approach gives no idea of pulsatile nature of $PGF_{2\alpha}$ release, it has the further advantage of eliminating the risk of overlooking a peak. However, for detailed studies of $PGF_{2\alpha}$ release patterns, frequent or continuous (*Basu & Kindahl* 1987) blood sampling techniques remain indispensable.

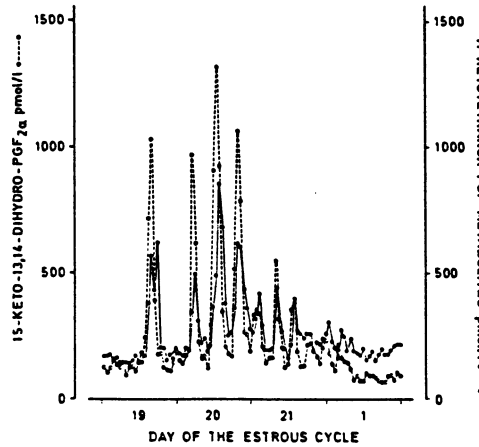

Figure 6. Peripheral plasma levels of 15- ketodihydro-$PGF_{2\alpha}$ (O - - - - O) and 11-ketotetranor PGF metabolites (●——●) during the luteolytic period in a heifer.

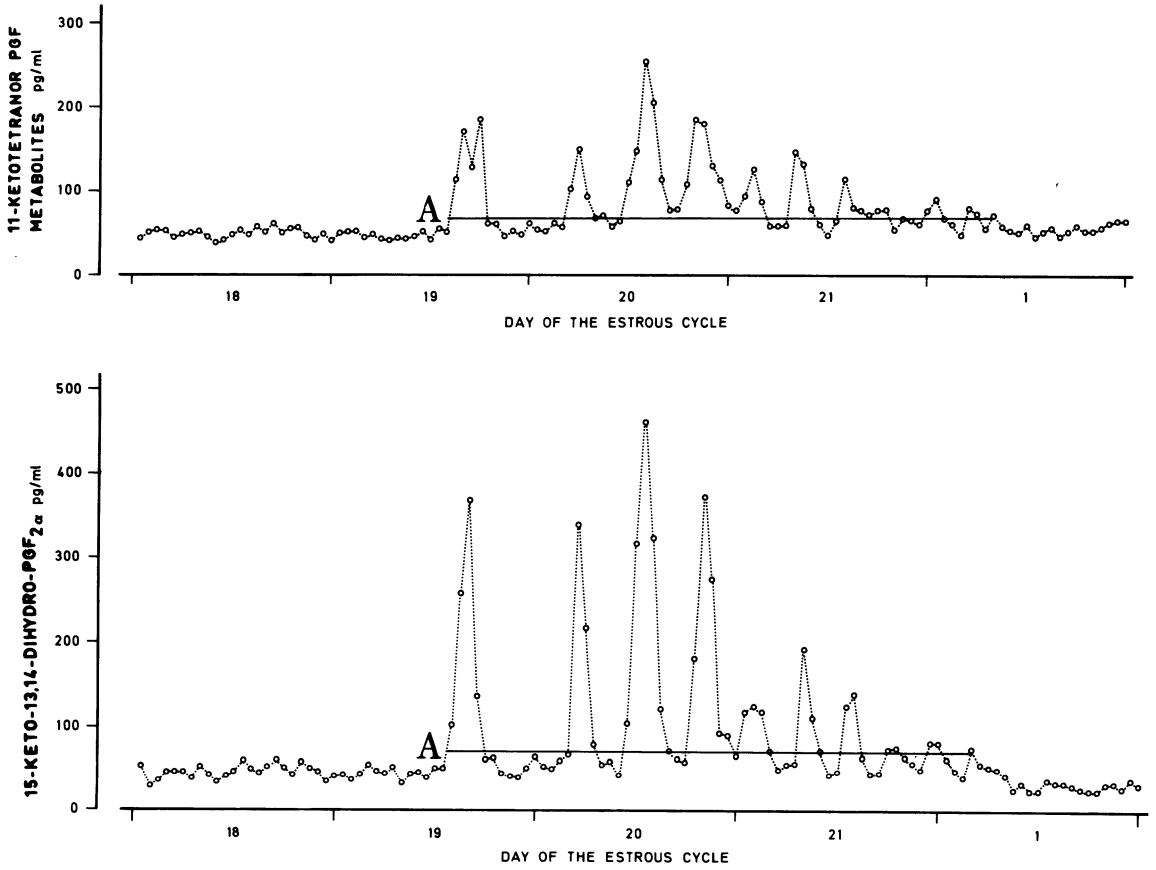

Figure 7. Peripheral plasma level of 11-ketotetranor PGF metabolites (upper panel) and 15-ketodihydro-$PGF_{2\alpha}$ (lower panel) during the luteolytic period in a heifer. The straight line A denotes the basal level + 2 SD.

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References

Basu S, Kindahl H, Harvey D, Goff A, Betteridge K: Blood plasma and urinary metabolites of $PGF_{2\alpha}$ release in the cattle. Proc. 10th. Int. Congr. Anim. Reprod. and A.I, June 1984, Illinois, 501.

Basu S, Kindahl H: Development of a continuous blood collection technique and a detailed study pf prostaglandin $F_{2\alpha}$ release during luteolysis and early pregnancy in heifers. J. Vet. Med. A 1987, *34,* 487-500.

Betteridge KJ, Eaglesome MD, Sugden EA, Mitchell D: Synchronization of estrus and ovulation in cattle with prostaglandin analouge AY 24655. Can. J. Anim. Sci. 1977, *57,* 23–32.

Betteridge KJ, Randall GCB, Eaglesome MD, Sugden EA: The influence of pregnancy on $PGF_{2\alpha}$ secretion in cattle. I. Concentrations of 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ and progesterone in peripheral blood of recipients of transferred embryos. Anim. Reprod. Sci. 1984, *7,* 195–216.

Goff AK, Basu S, Kindahl H: Measurement of 11-ketotetranor PGF metabolites: An approach for monitoring prostaglandin $F_{2\alpha}$ release in the mare. Theriogenology 1984, *21,* 887–896.

Granström E: On the metabolism of prostaglandin $F_{2\alpha}$ in female subjects. Structure of two metabolites in blood. Eur. J. Biochem 1972, *27,* 462–469.

Granström E, Kindahl H: Radioimmunoassay for urinary metabolites for prostaglandin $F_{2\alpha}$. Prostaglandins 1976, *12,* 759–783.

Granström E, Kindahl H: Species differences in circulating prostaglandin metabolites: Relevance for the assay of prostaglandin release. Biochim. Biophys. Acta 1982a, *713,* 555–569.

Granström E, Kindahl H: Radioimmunoassay of the major plasma metabolite of $PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$. Methods Enzymol. 1982b, *86,* 320–339.

Granström E, Kindahl H: Radioimmunoassay of 5α,7α-dihydroxy-11-ketotetranorprostane-1, 16-dioic acid, a major prostaglandin F metabolite in blood and urine. Methods Enzymol. 1982c, *86,* 339–353.

Granström E, Kindahl H, Swahn ML: Profiles of prostaglandin metabolites in the human circulation: Identification of late-appearing, long-lived products. Biochim. Biophys. Acta 1982, *713,* 46–60.

Hamberg M: Metabolism of prostaglandins in rat liver mitochondria. Eur. J. Biochem. 1968, *6,* 135–146.

Hamberg M, Samuelsson B: On the metabolism of prostaglandin E_1 and E_2 in man. J. Biol. Chem. 1971, *246,* 6713–6721.

Harvey D, Basu S, Betteridge KJ, Goff AK, Kindahl H: The influence of pregnancy on $PGF_{2\alpha}$ secretion in cattle. II. Urinary levels of 11-ketotetranor PGF metabolites and plasma progesterone concentrations during the oestrous cycle and early pregnancy. Anim. Reprod. Sci. 1984, *7,* 217–234.

Horton EW, Poyser NL: Uterine luteolytic hormone: A Physiological role for prostaglandin $F_{2\alpha}$. Physiol. Rev. 1976, *56,* 595–651.

Kindahl H, Edqvist L-E, Bane A, Granström E: Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ during the normal oestrous cycle and early pregnancy in heifers. Acta endocr. (Kbh.) 1976a, *82,* 134–149.

Kindahl H, Edqvist L-E, Granström E, Bane A: The release of prostaglandin $F_{2\alpha}$ as reflected by 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ in peripheral circulation during normal luteolysis in heifers. Prostaglandins 1976b, *11,* 871–878.

Samuelsson B: Quantitative aspects on prostaglandin synthesis in man. Advan. Biosci. 1974, *9,* 7–14.

Samuelsson B, Granström E, Greén K, Hamberg M, Hammarström S: Prostaglandins. Ann. Rev. Biochem. 1975, *44,* 669–695.

Samuelsson B, Goldyne M, Granström E, Hamberg M, Hammarström S, Malmsten C: Prostaglandins and Thromboxanes. Ann. Rev. Biochem. 1978, *47,* 997–1029.

Sammandrag

Prostaglandin $F_{2\alpha}$ metaboliter i blodplasma och urin som parametrar på prostaglandin $F_{2\alpha}$ frisättning hos kor.

Prostaglandin $F_{2\alpha}$ metaboliseras initialt till 15-ketodihydro-$PGF_{2\alpha}$ och vidare till 11-ketotetranor PGF metaboliter hos kor. Båda metaboliterna förekommer i den perifera blodcirkulationen och slutligen utsöndras 11-ketotetranor PGF metaboliterna i stora mängder till urinen i ett artspecifikt mönster. Det finns olika sätt för att detektera $PGF_{2\alpha}$ frisättning för studier inom reproduktionen: 1) analysera 15-ketodihydro-$PGF_{2\alpha}$ i perifer blodplasma; 2) analysera de mer långlivade 11-ketotetranor PGF metaboliterna i peri-

fer blodplasma; och 3) analysera 11-ketotetranor PGF metaboliterna i urin. Antikropparna som användes för radioimmunologiska analyser mot de olika prostaglandinmetaboliterna var specifika. Analysresultaten överensstämmer med tidigare gjorda masspektrometriska analyser. 11-Ketotetranor PGF analyser utfördes i urin från kor för att studera utsöndringen av prostaglandinmetaboliter efter i.v. infusion av $PGF_{2\alpha}$, under den normala brunstcykeln och den tidiga dräktighetsperioden. Studierna visar att 11-ketotetranor PGF metaboliterna utsöndrade till kourinen är en pålitlig parameter för detektering av exogent eller endogent $PGF_{2\alpha}$. För att mer i detalj kartlägga prostaglandinfrisättningen är det lämpligaste förfaringssättet att analysera 15-ketodihydro-$PGF_{2\alpha}$ metaboliten i frekvent insamlade blodprover.

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